

Interpretation of Preferential Interaction Coefficients of Nonelectrolytes and of Electrolyte Ions in Terms of a Two-Domain Model

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ABSTRACT For a three-component system consisting of solvent (1), polymer or polyelectrolyte (2J), and a nonelectrolyte or electrolyte solute (3), a two-domain description is developed to describe thermodynamic effects of interactions between solute components (2J) and (3). Equilibrium dialysis, which for an electrolyte solute produces the Donnan distribution of ions across a semipermeable membrane, provides a fundamental basis for this two-domain description whose applicability is not restricted, however, to systems where dialysis equilibrium is established. Explicit expressions are obtained for the solute-polymer preferential interaction coefficient $\Gamma_{3,2J}$ (nonelectrolyte case) and for $\Gamma_{+,2J}$ and $\Gamma_{-,2J}$, which are corresponding coefficients defined for single (univalent) cations and anions, respectively:

$$\Gamma_{+,2J} = |Z_J| + \Gamma_{-,2J} = 0.5(|Z_J| + B_{-,2J} + B_{+,2J}) - B_{1,2J}m_3/m_1$$

Here $B_{+,2J}$, $B_{-,2J}$, and $B_{1,2J}$ are defined per mole of species J, respectively, as the number of moles of cation, anion, and water included within the local domains that surround isolated molecules of J; Z_J is the charge on J; m_3 is the molal concentration of uniunivalent electrolyte, and $m_1 = 55.5$ mol/kg for water. Incorporating this result into a general thermodynamic description (derived by us elsewhere) of the effects of the activity a_{\pm} of excess uniunivalent salt on an equilibrium involving two or more charged species J (each of which is dilute in comparison with the salt) yields:

$$S_a K_{\text{obs}} \equiv d \ln K_{\text{obs}} / d \ln a_{\pm} = \Delta(\Gamma_{+,2J} + \Gamma_{-,2J}) = \Delta(B_{+,2J} + B_{-,2J} - 2B_{1,2J}m_3/m_1)$$

where K_{obs} is an equilibrium quotient defined in terms of the molar concentrations of the participants, J, and Δ denotes a stoichiometrically weighted combination of terms pertaining to the reactant(s) and product(s). The derivation presented here does not depend on any particular molecular model for salt-polyelectrolyte (or solute-polymer) interactions; it therefore generalizes our earlier (1978) derivation.

INTRODUCTION

Preferential interaction coefficients are fundamentally useful measures of the thermodynamic consequences of interactions involving solutes that participate in a reaction in solution. (Notation used in this paper to specify these coefficients is explained in the Appendix.) In a classic paper, Wyman (1964) used preferential interaction coefficients to analyze the effect of the activity a_3 of an uncharged solute (3) on an equilibrium involving one or more uncharged polymers (2J)

$$(\partial \ln K_{\text{obs}} / \partial \ln a_3)_{T,P} = \Delta \Gamma_{3,2J}. \quad (1)$$

Here K_{obs} is an equilibrium quotient (expressed in terms of the concentrations of the reactants and products) and the symbol Δ refers to the stoichiometrically weighted combination of preferential interaction coefficients pertaining to each of the participants 2J in the equilibrium: $\Gamma_{3,2J} \equiv (\partial m_3 / \partial m_{2J})_{T,\mu_1,\mu_3}$.

The derivation of Eq. (1) must be generalized to describe the effects of electrolyte activity on any type of equilibrium of charged (or uncharged) biopolymers, or the effects of uncharged solutes on equilibria involving charged polymers.

Anderson and Record (1993) showed that the effects of varying the mean ionic activity (a_{\pm}) of excess uniunivalent salt on an equilibrium involving one or more charged polymers can be described under typical conditions of experimental interest by the thermodynamic expression

$$S_a K_{\text{obs}} \equiv (\partial \ln K_{\text{obs}} / \partial \ln a_{\pm})_{T,P} = \Delta(|Z_J| + 2\Gamma_{3,2J}) \quad (2)$$

where Z_J is the charge of species J and $\Gamma_{3,2J}$ is the preferential interaction coefficient characterizing the interactions of uniunivalent salt with the electroneutral component 2J comprised of species J and a neutralizing complement of univalent salt ions. By expressing $|Z_J| + 2\Gamma_{3,2J}$ as a sum of single ion preferential interaction coefficients ($\Gamma_{+,2J} + \Gamma_{-,2J}$) that characterize the interactions of electrolyte cations and anions, respectively, with component 2J (Record and Richey, 1988), we show in the present paper that $S_a K_{\text{obs}} = \Delta(\Gamma_{+,2J} + \Gamma_{-,2J})$ and, hence, clarify the analogy between Eq. (1) and Eq. (2), the corresponding expression that pertains to charged solute components (2J and/or 3).

Previously we (Record et al., 1978) generalized the binding polynomial formulation of ligand effects on K_{obs} (cf. Wyman, 1964; Schellman, 1975) by incorporating electrolyte-charged polymer nonideality to analyze the typically profound effects of salt concentration (activity) on various kinds of equilibria involving charged biopolymers in water (specifically solubility, conformational changes, self-association, and ligand-binding). Application of these generalized binding polynomials to describe the thermodynamic

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effects of interactions between charged polymeric solutes and electrolyte ions, and introduction of the Gibbs-Duhem linkage of electrolyte and water activities, yield the following expression (in the present notation) for the dependence of K_{obs} on a_{\pm}

$$S_a K_{\text{obs}} = \Delta B_{+,2J} + \Delta B_{-,2J} - \frac{2m_3}{55.5} \Delta B_{\text{H}_2\text{O},2J} \quad (3)$$

Here $\Delta B_{+,2J}$, $\Delta B_{-,2J}$, and $\Delta B_{\text{H}_2\text{O},2J}$ represent stoichiometrically weighted differences among the numbers of cations, anions, and water, respectively, that are associated with products and/or reactants of the equilibrium for which K_{obs} is defined, and m_3 is the molal electrolyte concentration. In our previous papers, various symbols have been used instead of the $B_{i,J}$, which are in accord with notation currently in use, for example, by Timasheff (1992). (It should be noted that these thermodynamic binding coefficients are not directly related to second virial coefficients, often symbolized by B_i .) One important implication of Eq. (3) is that the cation and anion contributions to $S_a K_{\text{obs}}$ are additive (see also Record et al., 1976), a result in accord with the qualitative conclusions of numerous previous experimental investigations of the effects of electrolyte ions on biopolymer processes (reviewed and discussed by von Hippel and Schleich, 1969a, b; Record et al., 1978).

The derivation of Eq. (3) utilized the Gibbs-Duhem relation to obtain the contribution $\Delta B_{\text{H}_2\text{O},2J}$ to $S_a K_{\text{obs}}$ in a manner analogous to Tanford's (1969) analysis of the nonelectrolyte case, except that each mol of uniunivalent salt was recognized to contribute 2 mol of solute particles. Because our earlier derivation was based on a binding-polynomial formulation of ion-polyion interactions, it presupposed (at least formally) a set of ion and solvent binding equilibria for the formation of complexes having definite stoichiometries (Schellman, 1975). For applications of Eq. (3) to equilibria involving nucleic acids or other highly charged cylindrical polyions, limiting law expressions based on the hypothesis of counterion condensation (Manning, 1969) were incorporated to obtain an analytical stoichiometric interpretation of the effects of ion-polyelectrolyte nonideality on these equilibria. Some applications of Eq. (3) to interpret effects of salt concentration on solubility, conformational, self-association, and ligand-binding equilibria of proteins and nucleic acids have been provided previously (Record et al. 1978).

In the present paper, we develop a two-domain description based on dialysis equilibrium (Donnan equilibrium, in the case of charged solutes) to provide a physical interpretation of the terms comprising Eq. (3). This two-domain description of the distribution of water and ionic solutes interacting with a biopolymer is applied to interpret the single-ion preferential interaction coefficients ($\Gamma_{+,2J}$, $\Gamma_{-,2J}$) in terms of the accumulation or exclusion of cations and anions in a local domain surrounding each biopolymer. When applied to biopolymer processes, single-ion preferential interaction coefficients yield a general interpretation of $S_a K_{\text{obs}}$, which reduces to Eq.

(3) for the two-domain model of the solute distribution, and does not involve explicit application of the Gibbs-Duhem equation.

PREFERENTIAL INTERACTIONS OF NONELECTROLYTES

In a three-component system consisting of solvent (1), uncharged polymeric solute (2), and an uncharged low molecular weight solute, or a secondary solvent (3), the thermodynamic consequences of polymer-solute interactions are described by a preferential interaction coefficient $\Gamma_{3,2}$. (In this section, we consider only three-component systems consisting exclusively of uncharged species. Therefore, to simplify notation 2J is replaced by 2.) According to Eisenberg (1976), the preferential interaction coefficient for a three-component system can be defined as

$$\Gamma_{3,2} \equiv \left(\frac{\partial m_3}{\partial m_2} \right)_{T, \mu_1, \mu_3} \quad \lim_{m_2 \rightarrow 0} \Gamma_{3,2} \equiv \Gamma_{3,2}^{\circ} \quad (4)$$

where m_3 and m_2 are molal concentrations of solute and of polymer, respectively. The molal scale is more convenient than the molar scale for the present development. (In the Appendix, the results of this section are rederived using the molar concentration scale.)

Values of $\Gamma_{3,2}^{\circ}$ for nonelectrolytes have been interpreted in terms of a two-domain model (Inouye and Timasheff, 1972), in which the composition of the local region surrounding the polymer surface is compared with distant regions consisting entirely of "bulk" solution. More recently a 1:1 solute-solvent exchange model for discrete binding sites of solute and solvent on the polymer has been developed (Schellman, 1990, 1994; Timasheff, 1992). If a solute is preferentially accumulated near a polymer, relative to its concentration in bulk, $\Gamma_{3,2}^{\circ}$ is positive. Negative values of $\Gamma_{3,2}^{\circ}$ indicate preferential exclusion of solute (preferential solvation). For the (uncommon) quasi-ideal situation in which the solute and solvent are in effect randomly mixed, the local and bulk solute concentrations are identical, so that $\Gamma_{3,2}^{\circ} = 0$.

Equilibrium dialysis provides an experimental method of evaluating $\Gamma_{3,2}$ for both nonelectrolyte and electrolyte solutes by measurement of the difference in (diffusible) solute concentrations between two solutions separated by a semipermeable membrane. The solution designated β contains only membrane-permeable solute component (3) and solvent; the solution designated α also contains a membrane-impermeable component (2), which generally is of substantially higher molecular weight than either solvent or solute component 3. Here component 2 is referred to as a polymer, but the following development pertains equally well to systems where component 2 is an oligomer, or any other type of solute whose concentration is sufficiently dilute in comparison with component 3.

Provided that the osmotic pressure difference across the membrane at dialysis equilibrium is sufficiently small, the equilibrium condition can be expressed in terms of activities of the membrane-permeable solute component (rather than

chemical potentials)

$$a_{3(\alpha)} = a_{3(\beta)} \quad (5)$$

or

$$\gamma_{3(\alpha)} m_{3(\alpha)}^{\text{total}} = \gamma_{3(\beta)} m_{3(\beta)} \quad (6)$$

where $m_{3(\alpha)}^{\text{total}}$ is the total molal concentration of solute (3) in the polymer solution (α). The designation "total" is used to indicate that typically the sum of bound and free concentrations of solute is measured in the analytical assay (e.g., scintillation counting of a radiolabeled solute) applied at dialysis equilibrium.

The experimental thermodynamic distribution coefficient characterizing solute-polymer interactions at dialysis equilibrium is designated as $\Gamma_{3,2}^{\text{exp}}$

$$\Gamma_{3,2}^{\text{exp}} \equiv \frac{m_{3(\alpha)}^{\text{total}} - m_{3(\beta)}}{m_2} \quad (7)$$

From Eqs. 6–7

$$\Gamma_{3,2}^{\text{exp}} = \frac{((\gamma_{3,2})^{-1} - 1)m_{3(\beta)}}{m_2} \quad (8)$$

where

$$\gamma_{3,2} \equiv \gamma_{3(\alpha)}/\gamma_{3(\beta)} \quad (9)$$

may be interpreted as the contribution to nonideality of solute (3) arising from solute-polymer interactions. Eqs. (7–9) are, by definition, applicable to three-component nonelectrolyte solutions of any composition, in dialysis equilibrium with the corresponding two component solution. At sufficiently small values of m_2 , $\Gamma_{3,2}^{\text{exp}}$ ceases to be a function of m_2

$$\lim_{m_2 \rightarrow 0} \Gamma_{3,2}^{\text{exp}} \equiv \Gamma_{3,2}^{\circ} \quad (10)$$

Typically this limiting value of $\Gamma_{3,2}^{\circ}$ is attained at values of m_2 such that differences between $m_{3(\alpha)}$ and $m_{3(\beta)}$ can be accurately detected and $\Gamma_{3,2}^{\circ}$ can be evaluated as the slope of a linear plot of $m_{3(\alpha)}^{\text{total}}$ vs. m_2 , whose intercept is $m_{3(\beta)}$. Accumulation relative to solution β of solute (3) in the polymer-containing solution (α) results from preferential accumulation of solute (3) in the vicinity of polymer molecules; on the molal concentration scale, exclusion of solute (3) from the polymer-containing solution (equivalent to preferential accumulation of solvent) reflects exclusion of that solute from the vicinity of each polymer molecule (i.e., preferential solvation).

Consider the following two-domain model for a dilute polymer solution that contains one type of solute and solvent but is *not* necessarily in dialysis equilibrium. A "local" domain consisting exclusively of solute (3) and solvent surrounds each polymer molecule, and a "bulk" domain consisting of solute (3) and solvent separates all local domains. The term "local" implies that only one polymer affects the distribution of solute and solvent within its vicinity. To meet this condition, the solution must be sufficiently dilute in polymer to permit this division. By definition the local domains (con-

sidered together) contain $B_{1,2}$ mol solvent per mol polymer and $B_{3,2}$ mol solute (3) per mol polymer. The bulk region contains n_1^{bulk} mol solvent and n_3^{bulk} mol solute. In the polymer solution, the total molal concentration of solute (3) is

$$m_3^{\text{total}} = m_1 \frac{n_3^{\text{bulk}} + B_{3,2}n_2}{n_1^{\text{bulk}} + B_{1,2}n_2} \quad (11)$$

where m_1 is the fixed molal concentration of solvent. (For water, $m_1 = 55.5$ mol/kg.) The bulk concentration of solute(3), n_3^{bulk} , is given by

$$m_3^{\text{bulk}} = m_1 \frac{n_3^{\text{bulk}}}{n_1^{\text{bulk}}} \quad (12)$$

To proceed we require a thermodynamic criterion for distinguishing the local from the bulk domain. Consistent with the specification on the bulk domain given above, we postulate that the concentration of solute (3) in the bulk domain is the same as it would be in a polymer-free solution (β) in dialysis equilibrium with the polymer-containing solution (α)

$$m_{3(\alpha)}^{\text{bulk}} = m_{3(\beta)} \quad (13)$$

Eq. (13) is equivalent to an ideal dialysis equilibrium condition (cf. Eq. (6)). Therefore, from Eqs. (6), (9), and (13),

$$m_{3(\alpha)}^{\text{bulk}} = \gamma_{3,2} m_{3(\alpha)}^{\text{total}} \quad (14)$$

Equation (14) states that the contribution to solute (3) nonideality from solute-polymer interactions ($\gamma_{3,2}$) is accounted for entirely by the accumulation or exclusion (relative to the composition of the bulk solution) of solute (3) in the local domain surrounding each polymer; the activity of solute (3) in the bulk domain is assumed to be the same as it would be in solution β . Even in the absence of an actual dialysis equilibrium, the composition of the bulk domain in a sufficiently dilute three-component system can be *defined* as equal to that in the two-component system that *would be* in dialysis equilibrium with the solution of interest if a semipermeable membrane *were* introduced.

The molal concentration of polymer in compartment α is

$$m_2 = m_1 \frac{n_2}{n_{1(\alpha)}^{\text{bulk}} + B_{1,2}n_2} \quad (15)$$

Therefore from Eqs. (11) and (15)

$$\frac{m_{3(\alpha)}^{\text{total}}}{m_2} = \frac{n_3^{\text{bulk}} + B_{3,2}n_2}{n_2} \quad (16)$$

Substituting Eqs. (13–16) into Eq. (7), the following interpretation of $\Gamma_{3,2}^{\text{exp}}$ is obtained:

$$\Gamma_{3,2}^{\text{exp}} = B_{3,2} - B_{1,2} \frac{n_3^{\text{bulk}}}{n_{1(\alpha)}^{\text{bulk}}} = B_{3,2} - B_{1,2} \frac{m_{3(\alpha)}^{\text{bulk}}}{m_1} \quad (17)$$

Equation (17) is perfectly general with respect to solute concentrations, except that the solution must be sufficiently dilute in the polymer component (2) so that the osmotic pressure contribution in Eq. (5) can be neglected and so that a

bulk polymer-free phase can be defined. As $m_2 \rightarrow 0$,

$$\lim_{m_2 \rightarrow 0} \Gamma_{3,2}^{\text{exp}} = B_{3,2} - B_{1,2} \frac{m_3}{m_1} \equiv \Gamma_{3,2}^{\circ} \quad (18)$$

Equation (18) represents a novel derivation of the classic expression presented by Inouye and Timasheff (1972) for the nonelectrolyte case, which recently was derived by Schellman (1990) for a particular site-binding model of local solute-solvent exchange. This result also is consistent with that of Tanford's (1969) binding-polynomial analysis of effects of an uncharged solute on equilibria involving uncharged polymers.

DONNAN DIALYSIS EQUILIBRIUM OF A POLYELECTROLYTE: SINGLE-ION DONNAN COEFFICIENTS

The Donnan membrane equilibrium provides a fundamental measurement of the thermodynamics of interactions of ions with charged polymers. The experimental investigation of Donnan equilibrium is in many respects analogous to osmotic equilibrium and to equilibrium dialysis involving uncharged polymers and solutes. New effects arise, however, because of the charge on the membrane-permeable ionic solute(s) and on the membrane-impermeable charged polymer. At dialysis equilibrium of a polyelectrolyte-salt solution, these effects include: 1) equilibrium transmembrane differences in concentration of low molecular weight salt ions (M^+ and X^-), which together provide the most random mixture of ions consistent with the thermodynamic effects of interactions and with electroneutrality in the presence of a charged polymer; 2) an equilibrium membrane potential (the expression of small deviations from electroneutrality in each compartment resulting from transmembrane ion migration down the equilibrium concentration gradients of both cation and anion); and 3) an equilibrium osmotic pressure difference (the consequence of unequal concentrations of solutes on the two sides of membrane).

In analyses of the Donnan ion distribution that begin with the transmembrane equilibrium condition for the electroneutral salt component, it is generally acceptable to neglect the Donnan osmotic pressure difference and any macroscopic deviations from electroneutrality. As above, we designate the polyelectrolyte-containing solution with the subscript α , and the salt solution in dialysis equilibrium with it as β . If effects attributable to the Donnan osmotic pressure are neglected, the Donnan equilibrium condition is

$$a_{3(\alpha)}^{\text{eq}} = a_{3(\beta)}^{\text{eq}} \quad (19)$$

where, for a 1-1 electrolyte, $a_3 \equiv a_+ a_- \equiv \gamma_{\pm}^2 m_+ m_-$. Following the usual convention, the electroneutral polyelectrolyte component, $2J$, is defined to consist of a polyanionic species J and an equivalent number of univalent cations. As in the previous section, it suffices in this section (and in most of the following) to consider a general three-component system, so that to simplify notation the symbol $2J$ for the non-diffusible electroneutral component is replaced by 2. When

the concentration of salt is in excess of the concentration of polyanion charges, then to an excellent approximation the condition of electroneutrality can be applied to each solution

$$m_{+(\alpha)} = m_{-(\alpha)} + |Z_J| m_2 \quad \text{and} \quad m_{+(\beta)} = m_{-(\beta)} = m_{3(\beta)} \quad (20)$$

where Z_J is the valence of the polyanion. (The polycation case is obtained by replacing $|Z_J|$ by $-|Z_J|$.) By the definition of components,

$$m_{-(\alpha)} = m_{3(\alpha)} \quad (21)$$

From Eqs. (19–21), one obtains the usual Donnan result,

$$\gamma_{\pm(\beta)}^2 m_{3(\beta)}^2 = \gamma_{\pm(\alpha)}^2 m_{3(\alpha)} (m_{3(\alpha)} + |Z_J| m_2), \quad (22)$$

from which, at low m_2 , it follows that

$$m_{3(\beta)} \equiv \gamma_{3,2} (m_{3(\alpha)} + 0.5 |Z_J| m_2) \quad (23)$$

where (cf. Eq. 9)

$$\gamma_{3,2} \equiv \gamma_{\pm(\alpha)} / \gamma_{\pm(\beta)} \quad (24)$$

In obtaining Eq. (23) from Eq. (22), truncation of the expansion of the radical requires excess salt ($m_{3(\alpha)} \gg |Z_J| m_2$). Here, as in the nonelectrolyte case, $\gamma_{3,2}$ may be interpreted as the contribution to the nonideality of the electrolyte in compartment α arising from ion-polyion interactions.

The experimental thermodynamic salt-distribution coefficient $\Gamma_{3,2}^{\text{exp}}$ (the Donnan coefficient), which characterizes nonideality due to electrolyte-polyelectrolyte interactions, is defined by analogy to Eq. (7) for the nonelectrolyte case

$$\Gamma_{3,2}^{\text{exp}} \equiv \frac{m_{3(\alpha)} - m_{3(\beta)}}{m_2} = \frac{m_{-(\alpha)} - m_{-(\beta)}}{m_2} \equiv \Gamma_{-2}^{\text{exp}} \quad (25)$$

where

$$\lim_{m_2 \rightarrow 0} \Gamma_{3,2}^{\text{exp}} \equiv \Gamma_{3,2}^{\circ} = \lim_{m_2 \rightarrow 0} \left(\frac{\partial m_3}{\partial m_2} \right)_{T, \mu_1, \mu_3} \quad (26)$$

From Eqs. (21–23)

$$\Gamma_{-2}^{\text{exp}} = \frac{m_{3,\beta}}{m_2} ((\gamma_{3,2})^{-1} - 1) - 0.5 |Z_J|. \quad (27)$$

In the ideal limit of Eq. (27) where $\gamma_{3,2} = 1$, $\Gamma_{-2}^{\text{ideal}} = -0.5 |Z_J|$. (The ideal value of the Donnan coefficient may be approached in solutions containing weakly charged polymers at sufficiently low salt concentrations.) Generally, $\gamma_{3,2} < 1$ (i.e., the net effect of ion-polyion interactions is favorable) and $\Gamma_{-2}^{\circ} > -0.5 |Z_J|$.

For the counterion (here a positive ion)

$$\Gamma_{+2}^{\text{exp}} \equiv \frac{m_{+(\alpha)} - m_{+(\beta)}}{m_2} = 0.5 |Z_J| + \frac{m_{3(\beta)}}{m_2} ((\gamma_{3,2})^{-1} - 1) \quad (28)$$

where $\lim_{m_2 \rightarrow 0} \Gamma_{+2}^{\text{exp}} \equiv \Gamma_{+2}^{\circ}$. In the ideal limit where $\gamma_{3,2} = 1$, $\Gamma_{+2}^{\text{ideal}} = 0.5 |Z_J|$. Generally, $|Z_J| \geq \Gamma_{+2}^{\circ} \geq 0.5 |Z_J|$.

Counterion accumulation in and coion exclusion from the polyanion-containing compartment must be considered relative to the above-mentioned ideal reference state (most random electroneutral mixture). Note that, by conservation of material, $\Gamma_{+,2}^{\circ}$ and $\Gamma_{-,2}^{\circ}$ are always related:

$$\Gamma_{+,2}^{\circ} = |Z_2| + \Gamma_{-,2}^{\circ}. \quad (29)$$

TWO-DOMAIN INTERPRETATION OF $\Gamma_{-,2}$ AND $\Gamma_{+,2}$ FOR ELECTROLYTE-POLYELECTROLYTE (OR POLYAMPHOLYTE) INTERACTIONS

Our goal here is to interpret the single ion interaction (distribution) coefficients $\Gamma_{-,2}$ and $\Gamma_{+,2}$ using a two-domain (local-bulk) model of the electrolyte-polyelectrolyte solution. These coefficients are defined by Eqs. (27) and (28). By analogy to Eq. (11) for the nonelectrolyte case, the number of mols of cations ($B_{+,2}$) and anions ($B_{-,2}$) associated per mol of polyion in the two-domain model are related to total and bulk quantities by

$$\frac{m_{+,(\alpha)}^{\text{total}}}{m_2} = B_{+,2} + \frac{n_{+,(\alpha)}^{\text{bulk}}}{n_2} \quad \text{and} \quad \frac{m_{-,(\alpha)}^{\text{total}}}{m_2} = B_{-,2} + \frac{n_{-,(\alpha)}^{\text{bulk}}}{n_2} \quad (30)$$

where, by analogy to Eq. (12),

$$n_{+,(\alpha)}^{\text{bulk}} = \frac{n_{1(\alpha)}^{\text{bulk}} m_{+,(\alpha)}^{\text{bulk}}}{m_1} \quad \text{and} \quad n_{-,(\alpha)}^{\text{bulk}} = \frac{n_{1(\alpha)}^{\text{bulk}} m_{-,(\alpha)}^{\text{bulk}}}{m_1} \quad (31)$$

and, by analogy to Eq. (15),

$$n_2 = n_{1(\alpha)}^{\text{bulk}} m_2 (m_1 - B_{1,2} m_2)^{-1} \quad (32)$$

Eqs. (30–32) yield

$$\frac{m_{+,(\alpha)}^{\text{total}}}{m_2} = B_{+,2} + \frac{m_{+,(\alpha)}^{\text{bulk}}}{m_2} \left(1 - \frac{B_{1,2} m_2}{m_1} \right) \quad (33)$$

and

$$\frac{m_{-,(\alpha)}^{\text{total}}}{m_2} = B_{-,2} + \frac{m_{-,(\alpha)}^{\text{bulk}}}{m_2} \left(1 - \frac{B_{1,2} m_2}{m_1} \right).$$

Relationships between $m_{+,(\beta)}$ (or $m_{-,(\beta)}$) and $m_{+,(\alpha)}^{\text{bulk}}$ (or $m_{-,(\alpha)}^{\text{bulk}}$) are required. In the nonelectrolyte case (cf. Eqs. 13–14), the local-bulk model postulated that the contribution to nonideality of solute (3) arising from solute-polymer interactions was described entirely by the extent of accumulation of solute (3) in the local domain and that the bulk molal concentration of solute in the polymer-containing solution (α) was therefore equal to the solute concentration in the reference solution at dialysis equilibrium. For the electrolyte case, neither the local nor bulk phase in the polyelectrolyte-containing solution (α) is electroneutral. The bulk concentrations of cation and anion ($m_{+,(\alpha)}^{\text{bulk}}$ and $m_{-,(\alpha)}^{\text{bulk}}$) differ from one another and from their (equal) concentrations in the electroneutral reference compartment (β). By analogy with the definition of the bulk phase in the nonelectrolyte case (Eq. 13), we require that the ion concentrations in the bulk phase in α and in the “reference” solution (β) be related by an ideal

Donnan condition (i.e., $\gamma_{3(\alpha)}^{\text{bulk}} = \gamma_{3(\beta)}$) (cf. Eq. (22–24))

$$m_{3(\beta)}^2 = (m_{+,(\alpha)}^{\text{bulk}})(m_{-,(\alpha)}^{\text{bulk}}) \quad (34)$$

(In this thermodynamic two-domain model, the contribution to nonideality from electrolyte-polyelectrolyte interactions ($\gamma_{3,2}$; cf. Eq. 24) is interpreted entirely in terms of accumulation of cations (and/or anions) in the local domain surrounding the polyion.)

To proceed, we relate $m_{+,(\alpha)}^{\text{bulk}}$ to $m_{-,(\alpha)}^{\text{bulk}}$ (in Eq. 34) by an electroneutrality condition, initially expressed most conveniently in terms of mol numbers in the local and bulk regions

$$n_{+,(\alpha)}^{\text{bulk}} + n_{+,(\alpha)}^{\text{loc}} = n_{-,(\alpha)}^{\text{bulk}} + n_{-,(\alpha)}^{\text{loc}} + |Z_1| n_2 \quad (35)$$

where

$$n_{+,(\alpha)}^{\text{loc}} \equiv B_{+,2} n_2 \quad \text{and} \quad n_{-,(\alpha)}^{\text{loc}} \equiv B_{-,2} n_2.$$

Therefore,

$$n_{+,(\alpha)}^{\text{bulk}} - n_{-,(\alpha)}^{\text{bulk}} = (|Z_1| + B_{-,2} - B_{+,2}) n_2 \quad (36)$$

and

$$m_{+,(\alpha)}^{\text{bulk}} - m_{-,(\alpha)}^{\text{bulk}} = m_2 (|Z_1| + B_{-,2} - B_{+,2}) \left(1 - \frac{B_{1,2} m_2}{m_1} \right)^{-1}. \quad (37)$$

From Eqs. (35) and (37), after expansion of the radical for the condition of excess salt [i.e., $m_{+,(\alpha)}^{\text{bulk}}, m_{-,(\alpha)}^{\text{bulk}} \gg m_2 (|Z_1| + B_{-,2} - B_{+,2}) (1 - B_{1,2} m_2 / m_1)^{-1}$]

$$m_{+,(\beta)} = m_{+,(\alpha)}^{\text{bulk}} - 0.5 m_2 (|Z_1| + B_{-,2} - B_{+,2}) \left(1 - \frac{B_{1,2} m_2}{m_1} \right)^{-1} \quad (38)$$

$$m_{-,(\beta)} = m_{-,(\alpha)}^{\text{bulk}} + 0.5 m_2 (|Z_1| + B_{-,2} - B_{+,2}) \left(1 - \frac{B_{1,2} m_2}{m_1} \right)^{-1}$$

Evaluation of $\Gamma_{+,2}$ and $\Gamma_{-,2}$ (from Eqs. 25, 28, 33, and 38) yields

$$\Gamma_{+,2} = B_{+,2} - B_{1,2} \frac{m_{+,(\alpha)}^{\text{bulk}}}{m_1} + 0.5 (|Z_1| + B_{-,2} - B_{+,2}) \left(1 - \frac{B_{1,2} m_2}{m_1} \right)^{-1} \quad (39)$$

$$\Gamma_{-,2} = B_{-,2} - B_{1,2} \frac{m_{-,(\alpha)}^{\text{bulk}}}{m_1} - 0.5 (|Z_1| + B_{-,2} - B_{+,2}) \left(1 - \frac{B_{1,2} m_2}{m_1} \right)^{-1}$$

Equations (39) are consistent with the relationship $\Gamma_{+,2} = |Z_1| + \Gamma_{-,2}$ (cf. Eq. 29).

In the limit of low m_2 , where $m_{+,(\alpha)}^{\text{bulk}} = m_{-,(\alpha)}^{\text{bulk}} = m_3$, Eqs. (39) reduce to

$$\Gamma_{+,2}^{\circ} = 0.5 (|Z_1| + B_{-,2} + B_{+,2}) - B_{1,2} m_3 / m_1 \quad (40)$$

$$\Gamma_{-,2}^{\circ} = -0.5 (|Z_1| - B_{-,2} - B_{+,2}) - B_{1,2} m_3 / m_1 = \Gamma_{3,2}^{\circ}$$

Equations (40) indicate that the thermodynamic two-domain model of ion-polyion nonideality describes the difference between $\Gamma_{+,2}^\circ$ and its ideal value ($\Gamma_{+,2}^{\text{ideal}} = +0.5 |Z_J|$, cf. Eq. 28) in terms of: i) the modification of the polyion charge by the net effect of binding anions and cations; and ii) a hydration term, which contributes significantly only at moderate to high salt concentration. The molecular picture implied by this two-domain result, although certainly not literally descriptive of the interactions of ions with highly charged polyions, may be quite appropriate for weak interactions of ions with proteins, such as those responsible for Hofmeister effects. The entire thermodynamic manifestation of the steep radial gradients in the concentrations of cations and anions surrounding a highly charged polyion is modeled in terms of the ideal Donnan behavior of the polyion with a modified charge ($|Z_J| + B_{-,2} - B_{+,2}$). In either case, the two-domain picture should be a useful thermodynamic framework into which explicit calculations based on particular molecular models of ion-polymer interactions can be introduced.

Each term in the expression for $S_a K_{\text{obs}}$ given by Eq. (2) can be represented by summing the two Eqs. (40)

$$|Z_J| + 2\Gamma_{3,2}^\circ = \Gamma_{+,2}^\circ + \Gamma_{-,2}^\circ = B_{+,2} + B_{-,2} - \frac{2B_{1,2}m_3}{m_1}. \quad (41)$$

Restoring now the index J to differentiate the participants in the biopolymer equilibrium

$$\begin{aligned} \frac{d \ln K_{\text{obs}}}{d \ln a_{\pm}} &\equiv S_a K_{\text{obs}} = \Delta(\Gamma_{+,2J} + \Gamma_{-,2J}) \\ &= \Delta \left(B_{+,2J} + B_{-,2J} - \frac{2m_3}{m_1} B_{1,2J} \right) \end{aligned} \quad (42)$$

both $\Gamma_{+,2J}$ and $\Gamma_{-,2J}$, as well as their sum in Eq. (41) depend on both $B_{+,2J}$ and $B_{-,2J}$.

As an interesting special case of the above analysis, the polymer may be uncharged ($|Z_J| = 0$) but the cation and anion of the electrolyte interact differently with the polymer, so that $B_{+,2J} \neq B_{-,2J}$. Then the analogs of Eqs. (40) and (41) are $\Gamma_{+,2}^\circ = 0.5 (B_{-,2} + B_{+,2}) - B_{1,2}m_3/m_1 = \Gamma_{-,2}^\circ = \Gamma_{3,2}^\circ$ and $\Gamma_{+,2}^\circ + \Gamma_{-,2}^\circ = B_{-,2} + B_{+,2} - 2B_{1,2}m_3/m_1$.

COMPARISON WITH THE SOLUTE-SOLVENT EXCHANGE MODEL OF SCHELLMAN (1990)

Schellman (1990) developed a molecular description of preferential interactions, based on solute (3)-solvent (1) exchange with unit stoichiometry at discrete (identical, independent) sites on the polymer. For each class of sites b , an exchange equilibrium constant is defined as

$$K_b = \frac{\theta_{3b} a_1}{\theta_{1b} a_3} \quad (43)$$

where θ_{3b} and θ_{1b} represent fractional occupancies of the site

by solute and solvent, respectively ($\theta_{3b} + \theta_{1b} = 1$) and a_1 and a_3 are activities on the mol fraction scale. On the molal scale, the more conventional equilibrium constant K_b'' is

$$K_b'' \equiv K_b \frac{f_3}{f_1 m_1} = \frac{\theta_{3b}}{1 - \theta_{3b}} \frac{1}{m_3} \quad (44)$$

In Eq. (44), f_1 and f_3 are activity coefficients on the mol fraction scale. In water, $m_1 = 55.5$ mol/kg. For a nonelectrolyte solute and uncharged polymer, Schellman finds that the preferential interaction coefficient $\Gamma_{3,2}$ on the molal scale (his $\Gamma'_{3,2}$) is given generally by a sum of contributions Γ_{3b} at each class of sites

$$\Gamma_{3,2} = \sum_{b=1}^n \Gamma_{3b} \quad (45)$$

where each of the preferential interaction coefficients can be expressed explicitly

$$\Gamma_{3b} = \frac{(K_b'' - 1/m_1)m_3}{1 + K_b''m_3}. \quad (46)$$

Inserting Eq. (44) into (46), one obtains

$$\Gamma_{3b} = \theta_{3b} - \frac{m_3}{m_1} \theta_{1b} = \theta_{3b} - \frac{m_3}{m_1} (1 - \theta_{3b}).$$

Hence, this is the direct analog of Eq. (18) for the nonelectrolyte case.

For an electrolyte as solute and a charged polymer of valence $|Z_J|$, Schellman defines individual ion-solvent exchange constants

$$K_+ \equiv \frac{\theta_{+b} a_1}{\theta_{1b} a_+}; \quad K_- \equiv \frac{\theta_{-b} a_1}{\theta_{1b} a_-} \quad (47)$$

and a composite ion-solvent exchange constant K_b

$$K_b a_{\pm} = K_+ a_+ + K_- a_- = \frac{(\theta_{+b} + \theta_{-b}) a_1}{\theta_{1b}} \quad (48)$$

where $\theta_{1b} = 1 - (\theta_{+b} + \theta_{-b})$. For this case, Schellman finds that on the molal scale

$$K_b'' = K_b \frac{f_{\pm}}{f_1 m_1} = \frac{\theta_{+b} + \theta_{-b}}{\theta_{1b}} m_3 \quad (49)$$

and

$$\Gamma_{3,2} = -\frac{|Z_J|}{2 + m_3 \beta_{33}} + \sum_{b=1}^n \left[\frac{K_b''/2 - 1/m_1 m_3}{1 + K_b'' m_3} \right] \quad (50)$$

where $\beta_{33} = (\partial \mu_3 / \partial m_3)_{T,P,m_2}$ represents small-ion non-ideality. Therefore,

$$\Gamma_{3,2} = -\frac{|Z_J|}{2 + m_3 \beta_{33}} + \sum_{b=1}^n \left[\frac{\theta_{+b} + \theta_{-b}}{2} - \frac{m_3}{m_1} \theta_{1b} \right] \quad (51)$$

Neglecting the β_{33} term, we see that

$$|Z_J| + 2\Gamma_{3,2} = \sum_{b=1}^n \left[\theta_{+b} + \theta_{-b} - \frac{2m_3}{m_1} \theta_{1b} \right] \quad (52)$$

Equation (52) is the analog of our result (Eq. 41) for the electrolyte case, and yields an expression for $S_a K_{\text{obs}}$ analogous to that originally derived by Record et al. (1978) (cf. Eq. (42) above). Schellman's (1990) formulation provides an interpretation of the quantities $B_{+,2}$ and $B_{-,2}$ (relative to $B_{1,2}$) in terms of the equilibrium constant K_b'' (Eqs. 47–49) for ion-solvent exchanges. Schellman's approach, based on these exchange reactions, is fundamentally distinct from ours, which is based on local-bulk domain partitioning and does not appeal to discrete sites or a specified exchange stoichiometry at those sites.

Timasheff (1992) explicitly treated the limiting case where some sites on the protein participate in solute-solvent exchange and others do not. For N_1 exchangeable sites and N_2 nonexchangeable (solvated) sites

$$\Gamma_{3,2} = N_1 \theta_{3b} - \frac{m_3}{m_1} (N_1 \theta_{3b} + N_2). \quad (53)$$

Comparison of Eq. (53) with Eq. (18) for the nonelectrolyte case indicates that $B_{3,2} = N_1 \theta_{3b}$ and $B_{1,2} = N_2 + N_1(1 - \theta_{3b})$.

GENERALIZATION OF THE SOLUTE-SOLVENT EXCHANGE MODEL

As noted by Schellman (1990), the molar amounts of solute and solvent in the local region surrounding a polymer may be related by the stoichiometry of solute-solvent exchange. A generalization of Schellman's concept may be used to recast Eq. (18) for the nonelectrolyte case in terms of $B_{1,2}^0$, the extent of solvation of the polymer in the absence of solute and a cumulative stoichiometry $S_{1,3}$ of solvent displacement upon solute accumulation

$$S_{1,3} \equiv (B_{1,2}^0 - B_{1,2})/B_{3,2}. \quad (54)$$

Hence, Eq. (18) becomes

$$\Gamma_{3,2}^0 = B_{3,2} \left(1 + \frac{m_3}{m_1} S_{1,3} \right) - \frac{m_3}{m_1} B_{1,2}^0. \quad (55)$$

For small solutes, $S_{1,3}$ is expected to be near 1, so the term $m_3 S_{1,3}/m_1$ will typically be significant only at solute concentrations in excess of 1 molal. If a particular functional form of the dependence of $B_{3,2}$ on m_3 is assumed (e.g., site binding, phase partitioning, adsorption, counterion condensation), then Eq. (55) may be compared with experimental data, which typically indicate that $\Gamma_{3,2}$ for nonelectrolytes is linear in m_3 when $m_3 \leq 1$ molal (e.g., Timasheff, 1992).

For the electrolyte case, incorporating cumulative stoichiometries $S_{1,+}$ and $S_{1,-}$ of solvent displacement upon cation

and anion accumulation yields

$$B_{1,2} = B_{1,2}^0 - (S_{1,-})B_{-,2} - (S_{1,+})B_{+,2} \quad (56)$$

so that Eqs. (40) and (41) become

$$\Gamma_{+,2}^0 = 0.5 \left[|Z_J| + B_{-,2} \left(1 + \frac{(S_{1,-})m_3}{m_1} \right) + B_{+,2} \left(1 + \frac{(S_{1,+})m_3}{m_1} \right) \right] - \frac{B_{1,2}^0 m_3}{m_1} \quad (57)$$

$$\Gamma_{-,2}^0 = \Gamma_{3,2}^0 = -0.5 \left[|Z_J| - B_{-,2} \left(1 + \frac{(S_{1,-})m_3}{m_1} \right) - B_{+,2} \left(1 + \frac{(S_{1,+})m_3}{m_1} \right) \right] - \frac{B_{1,2}^0 m_3}{m_1}$$

and

$$\Gamma_{+,2}^0 + \Gamma_{-,2}^0 = B_{+,2} \left(1 + \frac{(S_{1,+})m_3}{m_1} \right) + B_{-,2} \left(1 + \frac{(S_{1,-})m_3}{m_1} \right) - \frac{2B_{1,2}^0 m_3}{m_1}. \quad (58)$$

TWO-DOMAIN EXPRESSIONS FOR THE EXCESS FREE ENERGY ($RT \ln \gamma_{3,2}$)

Although our primary focus in this paper has been on $\Gamma_{3,2}$ because of its direct relevance to the analysis of $(\partial \ln K_{\text{obs}} / \partial \ln a_3)_{T,P}$, it is of general interest to obtain $\gamma_{3,2}$ and an expansion of the excess free energy ($RT \ln \gamma_{3,2}$) attributable to solute (3)-polymer (2) interactions in the context of the two-domain model.

From Eq. (8)

$$(\gamma_{3,2})^{-1} = 1 + m_2 \Gamma_{3,2} / m_{3(\beta)}. \quad (59)$$

In the two-domain interpretation of Eq. (59)

$$(\gamma_{3,2})^{-1} = 1 + m_2 ((B_{3,2}/m_{3(\beta)}) - (B_{1,2}/m_1)). \quad (60)$$

The excess free energy (in units of RT) is

$$-\ln \gamma_{3,2} = \ln \left(1 + \frac{m_2 \Gamma_{3,2}}{m_{3(\beta)}} \right) \approx \frac{m_2 \Gamma_{3,2}}{m_{3(\beta)}} + 0.5 \left(\frac{m_2 \Gamma_{3,2}}{m_{3(\beta)}} \right)^2 + \dots \quad (61)$$

In the two-domain model, at low m_2

$$-\ln \gamma_{3,2} \approx \frac{m_2 \Gamma_{3,2}}{m_{3(\beta)}} = m_2 \left(\frac{B_{3,2}}{m_{3(\beta)}} - \frac{B_{1,2}}{m_1} \right) \quad (62)$$

In the (quasi-) ideal situation, where $B_{3,2}/B_{1,2} = m_{3,\beta}/m_1$, then $\gamma_{3,2} = 1$, $\ln \gamma_{3,2} = 0$, and $\Gamma_{3,2} = 0$. If the polymer is preferentially hydrated, so $B_{3,2} = 0$ at all $m_{3,\beta}$, then $\gamma_{3,2} = (1 - B_{1,2} m_2 / m_1)^{-1}$, $\ln \gamma_{3,2} \approx B_{1,2} m_2 / m_1 > 0$, and $\Gamma_{3,2} = -B_{1,2} m_{3(\beta)} / m_1 < 0$. If, on the other hand, the solute is very strongly accumulated, so $B_{3,2}/m_{3(\beta)} \gg B_{1,2}/m_1$, then $\gamma_{3,2} = (1 + B_{3,2} m_2 / m_{3(\beta)})^{-1}$, $\ln \gamma_{3,2} \approx -B_{3,2} m_2 / m_{3(\beta)}$, and $\Gamma_{3,2} = B_{3,2} > 0$.

From Eq. (27) the contribution to solute (3) nonideality from solute-polyion interactions in the electrolyte-polyelectrolyte (or polyampholyte) case, is

$$\begin{aligned}\gamma_{3,2}^{-1} &= 1 + \frac{m_2}{m_{3(\beta)}} (0.5 |Z_J| + \Gamma_{3,2}) \\ &= 1 + m_2 \left(\frac{B_{+,2} + B_{-,2}}{2m_{3(\beta)}} - \frac{B_{1,2}}{m_1} \right)\end{aligned}\quad (63)$$

The excess free energy $\ln \gamma_{3,2}$, at low m_2 , is

$$-\ln \gamma_{3,2} \cong m_2 \frac{(B_{+,2} + B_{-,2})}{2m_{3(\beta)}} - \frac{B_{1,2}}{m_1}\quad (64)$$

In this case, the criterion for ideal behavior is that

$$\left[\frac{B_{+,2} + B_{-,2}}{B_{1,2}} \right]_{\text{ideal}} = \frac{2m_{3(\beta)}}{m_1}\quad (65)$$

Net electrolyte accumulation or exclusion from the local domain is quantified relative to this (quasi-) ideal condition.

CONCLUDING REMARKS

The explicit formalism recently developed by Schellman (1990) and Timasheff (1992) to describe preferential interactions of solute and solvent with a polymer presupposes a definite molecular picture for the solvent-solute exchange. In contrast to these and to our own previous work (Record et al., 1978), the two-domain approach developed here is not dependent on site-binding, counterion condensation, or any other specific description at the molecular level. Instead, we have proposed a more general thermodynamic basis for the definition of a local domain whose consequences have been explicitly derived here in terms of expressions describing the preferential interactions of nonelectrolyte solutes or, in particular, of individual ions with a charged (or uncharged) polymer. This two-domain approach can supply the framework within which more detailed descriptions of ion-polyion interactions can be constructed in order to arrive at quantitative predictions for the characteristic parameters ($B_{i,2}$).

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APPENDIX

Labeling convention for preferential interaction coefficients

It suffices for most of the development presented in this paper to consider a three-component system comprised of principal solvent (component "1") and two types of solute components, one of which can be classified as "diffusible" and the other as "nondiffusible" (regardless of whether a dialysis equilibrium is actually established). Compared with the "nondiffusible" solute, the "diffusible" solute is of relatively low molecular weight and in relatively large excess. Following tradition (cf. Eisenberg, 1976) we label

the "diffusible" solute component as "3". This component can be either a simple electrolyte (such as NaCl) or nonelectrolyte (such as glycerol). We also retain the convention of using the label "2" in referring to the nondiffusible solute component, which can be either a nonelectrolyte or an oligo- or polyelectrolyte component, comprised of a multiply charged species and the requisite number of oppositely charged salt ions. Thermodynamic effects of the interactions of this component with the other solute (3) can be described by the preferential interaction coefficient $\Gamma_{3,2}$, defined as a derivative expressing how changes in the concentration of component 3 depend upon changes in the concentration of component 2, under the specified constraints. (Cf. Eq. 4).

All of the systems considered in this paper contain a single type of "diffusible" component (3) present in large excess. To describe the effects of varying the activity of this component on an equilibrium involving at least two distinct types of nondiffusible species, it is practically necessary (for the sake of clarity) to adopt a labeling system that indicates the existence of more than one type of nondiffusible solute in the solution. For this purpose we introduced in our previous paper (Anderson and Record, 1993) the symbol 2J. The numerical index 2 is retained to emphasize that all participants in the equilibrium of interest may be regarded as nondiffusible for the purpose of defining the preferential interaction coefficients $\Gamma_{3,2J}$ that appear in the expression we derived for $S_A K_{\text{obs}}$. (Even though there are more than two types of solute components in the system, three-component preferential interaction coefficients of the form $\Gamma_{3,2J}$ suffice for this analysis, because by assumption the nonideality of each of the components 2J is determined exclusively by its interactions with excess solute component 3 and the solvent.) The symbol J, which should not be viewed as representative of a numerical index, stands for any of the (nondiffusible) participants in the equilibrium of interest. If these reactants and products are charged, then J denotes any of the species actually involved in the equilibrium, whereas 2J denotes the corresponding electroneutral component. Both species and components enter explicitly into the derivation of the expression for $S_A K_{\text{obs}}$ pertaining to charged reactants and products (Anderson and Record, 1993). For a reactant (product) that is uncharged, "J" has no meaning apart from the symbol 2J, because for nonelectrolytes there is no distinction between species and components.

Two-domain analysis of $\Gamma_{3,2}$ defined in terms of molar concentrations

For nonelectrolytes, the preferential interaction coefficient measurable by dialysis equilibrium can be defined on the molar concentration scale as

$$\Gamma_{3,2}^{\text{exp}} \equiv \frac{C_{3(\alpha)}^{\text{total}} - C_{3(\beta)}}{C_2}\quad (A1)$$

where

$$C_{3(\alpha)}^{\text{total}} = \frac{B_3 n_2 + n_3^{\text{bulk}}}{V_\alpha}; \quad C_{3(\beta)} = \frac{n_{3(\beta)}}{V_\beta} = \frac{n_{3(\alpha)}^{\text{bulk}}}{V_\alpha^{\text{bulk}}}; \quad \text{and} \quad C_2 = \frac{n_2}{V_\alpha}.$$

Therefore

$$\frac{C_{3(\alpha)}^{\text{total}}}{C_2} = B_{3,2} + \frac{n_3^{\text{bulk}}}{n_2}\quad (A2)$$

and

$$\frac{C_{3(\beta)}}{C_2} = \frac{n_3^{\text{bulk}}}{V_\alpha^{\text{bulk}}} \frac{V_\alpha^{\text{loc}} + V_\alpha^{\text{bulk}}}{n_2} = \frac{n_3^{\text{bulk}}}{n_2} \left(1 + \frac{V_\alpha^{\text{loc}}}{V_\alpha^{\text{bulk}}} \right)\quad (A3)$$

because $V_\alpha = V_\alpha^{\text{loc}} + V_\alpha^{\text{bulk}}$.

Combining Eqs. (A1–A3), one obtains

$$\Gamma_{3,2}^{\text{exp}} = B_{3,2} - \frac{n_3^{\text{bulk}}}{n_2} \frac{V_\alpha^{\text{loc}}}{V_\alpha^{\text{bulk}}} = B_{3,2} - \frac{n_3^{\text{bulk}}}{V_\alpha^{\text{bulk}}} \frac{V_\alpha^{\text{loc}}}{n_2}\quad (A4)$$

Because

$$V_\alpha^{\text{loc}} = n_2 \bar{V}_2 + n_1^{\text{loc}} \bar{V}_1 + n_3^{\text{loc}} \bar{V}_3 = n_2 (\bar{V}_2 + B_{1,2} \bar{V}_1 + B_{3,2} \bar{V}_3)\quad (A5)$$

therefore

$$\begin{aligned}\Gamma_{3,2}^{\text{exp}} &= B_{3,2} - C_{3(\alpha)}^{\text{bulk}}(\bar{V}_2 + B_{1,2}\bar{V}_1 + B_{3,2}\bar{V}_3) \\ &= B_{3,2}(1 - C_{3(\alpha)}^{\text{bulk}}\bar{V}_3) - C_{3(\alpha)}^{\text{bulk}}\bar{V}_2 - C_{3(\alpha)}^{\text{bulk}}B_{1,2}\bar{V}_1\end{aligned}\quad (\text{A6})$$

Derivations analogous to that of Eq. (A6) may be used to obtain single-ion preferential interaction coefficients on the molar concentration scale.

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